

Case 21246 WO

claims (19.04.2004)

DT05 Rec'd PCT/PTO 14 OCT 2004

1. (Amended) A purified aldehyde dehydrogenase having the following physico-chemical properties:

- a) Molecular weight of $100,000 \pm 10,000$ Da (consisting of two homologous subunits) or molecular weight of $150,000 \pm 15,000$ Da (consisting of three homologous subunits), where each subunit has a molecular weight of $55,000 \pm 2,000$ Da;
- b) Substrate specificity: active on L-sorbosone, D-glucosone, D-glucose, D-xylose;
- c) Cofactor: pyrroloquinoline quinone (PQQ),
- d) Optimum pH of from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbosone) or optimum pH of about 9.0 (for the production of 2-keto-L-gulonic acid from L-sorbosone),
- e) Inhibitors: Co^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Zn^{2+} , and monoiodoacetate.

2. The aldehyde dehydrogenase according to claim 1, which is derived from a microorganism belonging to the genus *Gluconobacter* which is capable of producing said aldehyde dehydrogenase.

3. The aldehyde dehydrogenase according to claim 2, wherein the microorganism is *Gluconobacter oxydans* having the identifying characteristics of the strain *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

4. The aldehyde dehydrogenase according to claim 3, wherein the microorganism is *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

5. A process for producing an aldehyde dehydrogenase having the following physico-chemical properties:

- a) Molecular weight of $100,000 \pm 10,000$ Da (consisting of two homologous subunits) or molecular weight of $150,000 \pm 15,000$ Da (consisting of three homologous subunits), where each subunit has a molecular weight of $55,000 \pm 2,000$ Da;
- b) Substrate specificity: active on aldehyde compounds,
- c) Cofactor: pyrroloquinoline quinone (PQQ),
- d) Optimum pH of from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbosone) or optimum pH of about 9.0 (for the production of 2-keto-L-gulonic acid from L-sorbosone),
- e) Inhibitors: Co^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Zn^{2+} , and monoiodoacetate,

which comprises cultivating a microorganism belonging to the genus *Gluconobacter*, which is capable of producing the aldehyde dehydrogenase having the above properties, in an aqueous nutrient medium under aerobic conditions, disrupting the cells of the microorganism, and

isolating and purifying the aldehyde dehydrogenase from the cell-free extract of the disrupted cells of the microorganism.

6. The process according to claim 5, wherein the reaction is carried out at a pH of from about 5.5 to 9.0 and at a temperature of from about 20 to about 50°C.

7. A process for producing a carboxylic acid and/or its lactone from its corresponding aldose which comprises contacting the aldehyde with the purified aldehyde dehydrogenase having the following physico-chemical properties:

a) Molecular weight of $100,000 \pm 10,000$ Da (consisting of two homologous subunits) or molecular weight of $150,000 \pm 15,000$ Da (consisting of three homologous subunits), where each subunit has a molecular weight of $55,000 \pm 2,000$ Da;

b) Substrate specificity: active on aldehyde compounds,

c) Cofactor: pyrroloquinoline quinone (PQQ),

d) Optimum pH of from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbose) or optimum pH of about 9.0 (for the production of 2-keto-L-gulonic acid from L-sorbose),

e) Inhibitors: Co^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Zn^{2+} , and monooiodoacetate,

or cell-free extract prepared from a microorganism belonging to the genus *Gluconobacter* which is capable of producing the aldehyde dehydrogenase having the above properties in the presence of an electron acceptor.

8. The process according to claims 5 to 7, wherein the microorganism is *Gluconobacter oxydans* having the identifying characteristics of the strain *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

9. The process according to claim 8, wherein the microorganism is *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

10. The process of claim 7, wherein the lactone is vitamin C, the carboxylic acid is 2-keto-L-gulonic acid and the aldose is L-sorbose.

11. The process according to any one of claims 7 to 10, wherein the reaction is carried out at a pH of from about 5.5 to about 9.0 and at a temperature of from about 20 to about 50°C for the production of vitamin C and 2-keto-L-gulonic acid, respectively.

12. The process according to any one of claims 7 to 11, wherein the reaction is carried out at a pH of from about 6.5 to about 8.0 and a temperature of from about 20 to about 40°C for the

production of vitamin C, and at a pH of about 9.0 and a temperature of from about 20 to about 30°C for the production of 2-keto-L-gulonic acid.

13. The use of the purified aldehyde dehydrogenase of claim 1 in the process for the production of a carboxylic acid and/or its lactone from its corresponding aldose which comprises contacting the aldehyde with said purified aldehyde dehydrogenase or cell-free extract prepared from a microorganism belonging to the genus *Gluconobacter* which is capable of producing said aldehyde dehydrogenase in the presence of an electron acceptor.